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Comparison of scorpion behavioral responses to UV under sunset and nighttime irradiances

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Abstract. Scorpions are nocturnal arachnids that fluoresce a bright cyan-green when exposed to UV light. Although the function of this fluorescence remains unknown, some authors have suggested that it may aid the scorpions' light detection. Taking advantage of scorpions' negatively phototactic behavior, we tested the responses of desert grassland scorpions, *Paruroctonus utahensis* (Williams 1968), to 395 nm UV light at irradiances corresponding to an hour before sunset (0.15 μW/cm^2), sunset (0.01 μW/cm^2), and moonlight (0.0001 μW/cm^2), as well as no light. We found that animals showed the strongest responses to UV light levels equivalent to sunset. The animals moved more quickly and sporadically under the higher light levels. In addition, animals were less likely to complete a trial under highest light conditions, suggesting that UV light may inhibit normal scorpion locomotion. Finally, this study resulted in several methodological refinements, including automated tracking of the subjects' movements that should prove useful in future behavioral studies of scorpion phototactic behavior.

Keywords: Fluorescence, light, orientation, sensory, vision

Scorpions are nocturnal arachnids that fluoresce a bright green color when exposed to ultraviolet (UV) light due to the presence of beta-carboline and 4-methyl-7-hydroxycoumarin in their cuticle (Stachel et al. 1999; Frost et al. 2001). No functional reason behind their fluorescence has been determined. Some authors, including Frost et al. (2001) and Wankhede (2004), have suggested that scorpion fluorescence may serve no behavioral purpose, while others have proposed that fluorescence may help scorpions capture prey (Kloock 2005), attract mates, or ward off predators and territorial rivals (Kloock 2008). Other researchers hypothesize that fluorescence may play an active role in light detection, helping scorpions identify shelter or decide when to stay in their burrows (Camp & Gaffin 1999; Blass & Gaffin 2008; Gaffin et al. 2012; Kloock et al. 2010).

Scorpion cuticle fluoresces most strongly under 395 nm UV light, reemitting it as green (~ 500 nm: Fasel et al. 1997; Kloock 2009). Studies indicate that the medial eyes of scorpions are most sensitive to green light (peaking around 500 nm: Machan 1968; Fleissner & Fleissner 2001). Parts of the scorpion metasoma are also sensitive to green light (Zwicky 1968, 1970a,b; Rao & Rao 1973). It is therefore tempting to suggest that fluorescence may aid in light detection by transducing UV light to increase light intensity in the range of peak sensitivity of their visual system.

A few behavioral studies support this hypothesis. Blass & Gaffin (2008) showed that scorpions become most active when exposed to UV or green light, as compared to other wavelengths. Kloock et al. (2010) found a difference between fluorescent and fluorescence-reduced scorpions in the variance of time spent in light-exposed areas, as well as differences in activity levels under UV light. Gaffin et al. (2012) found that scorpions with medial and lateral eyes covered were far less likely to react to 505 nm light, but only slightly less likely to react to 395 nm light. This last study led to the hypothesis that the cuticle may act as a whole-body UV photon collector, transducing UV wavelengths to green wavelengths. This information may allow the scorpion to detect and turn toward shade when one part of the cuticle receives diminished light levels.

Taken together, the physiological and behavioral evidence suggest that UV light plays an important role in scorpion orientation. However, to better understand these effects, we first need to quantify the levels at which UV light becomes behaviorally relevant.

Our objective in the current study was to establish a dose response curve illustrating scorpions' reactions to irradiances of UV light corresponding to natural conditions ranging from early sunset to the middle of the night. Gaffin et al. (2012) observed significant phototactic behavior when scorpions were exposed to 0.15 μW/cm^2 UV light, slightly greater than the UV component of sunlight about an hour before sunset when the sun is 11.4° above the horizon (Johnsen et al. 2006). This light level is somewhat higher than what scorpions normally encounter; they become active shortly after sunset (Polis 1980) and are therefore most likely to encounter intensities of light corresponding to refracted sunlight, starlight, or moonlight.

We used 0.15 μW/cm^2 as the high light treatment on our dose response curve. We selected 0.01 μW/cm^2, the UV component of the sky's irradiance at sunset (when the sun is at the horizon: Johnsen et al. 2006), as the second treatment. We selected 0.0001 μW/cm^2, the UV component of full moonlight on a clear night (Johnsen et al. 2006), as the third and lowest light treatment. Finally, we used no light as the control and the final point on the curve, where we expected to see no phototactic behavior. Combined, these four points represented a relatively even distribution of celestial irradiance values that would be present from the early evening into the night.

We found that scorpions respond to UV light levels that correspond to irradiance values found around sunset. Since this assay uses a negative phototactic locomotor behavior as the response, the actual threshold of sensitivity is probably lower than these deterrence levels. Taken together, scorpions appear capable of detecting UV levels that are consistent with light levels during early evening.

An additional objective of this study was to improve the efficiency of the behavioral assay used to detect light avoidance behavior in scorpions. We have greatly improved

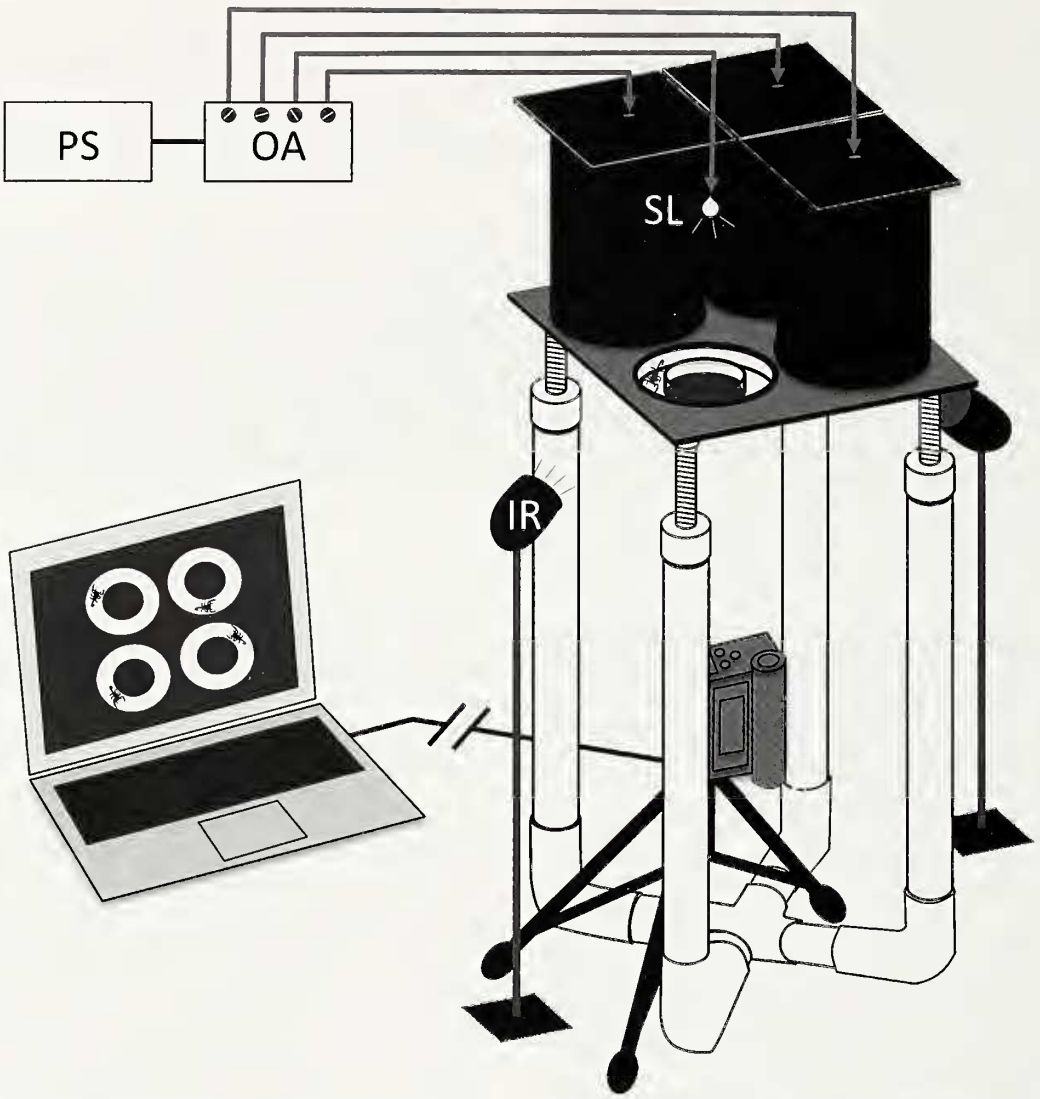

Figure 1.—Diagram of behavioral set-up. A power source (PS) powers four independently controllable operational amplifier circuits (OA), which connect to LEDs (SL) that extend through holes in the tops of dark PVC cylinders placed over Petri dish arena. An IR sensitive video camera below the stage monitors scorpion activity. Infrared light is direeted across the bottoms of the arenas from two sources (IR) placed at the side of the set-up. Video output is relayed to a computer for recording, processing, and analysis.

the visibility of the animals in the behavioral arenas and applied automated tracking software to assist in the scoring of behavioral trials and to reduce possible sources of human bias.

METHODS

Animals.—We used 12 male and 12 female adult *Paruroctonus utahensis* (Williams 1968) scorpions collected in late August and early September 2012 from sandy regions of the northern Chihuahua Desert. Collecting areas ranged from the Texas-New Mexico border between El Paso and Las Cruces to areas east of Socorro, New Mexico and near the Sevilleta field station in La Joya, New Mexico. We deposited a voucher specimen in the Sam Noble Oklahoma Museum of Natural History on the University of Oklahoma campus in Norman, Oklahoma. Animals were kept in the laboratory at the Sevilleta station and housed individually in plastic food storage containers (Great Value, 236 ml) that had four 5.6 mm air holes drilled in the corners of their lids. Each container also held 20 ml of sand from their native habitat (filtered through a #12 sieve) and a 4 cm × 4 cm square of paperboard folded into a tent for shelter. The animals were provided a few ml of water weekly by misting and a wax worm every other week. The animals were exposed to a 14:10 h light:dark cycle (on at 0530, off at 1930) using a white fluorescent bulb (General Electric "Energy Smart" 13 W bulb – 60 W equivalent) in a work light (Bayco clamp light, 21.6 cm) plugged into a timer switch. The light was placed 50 cm from the animals. The room temperature ranged from 20–21°C during the day. To increase animal activity on trial nights, a small heater (Sunbeam compact ceramic heater) was used to warm the room to 22–24°C.

Behavioral apparatus.—We used a modified version of the apparatus described in Gaffin et al. (2012). Figure 1 shows a diagram of the behavioral set-up. We created a circular arena for the animals by gluing a 5.40-cm diameter Petri dish upside down in the center of an 8.75-cm diameter Petri dish (15 mm deep). The larger Petri dish lids were used for covers and secured to the dish bottoms with small pieces of electrical tape.

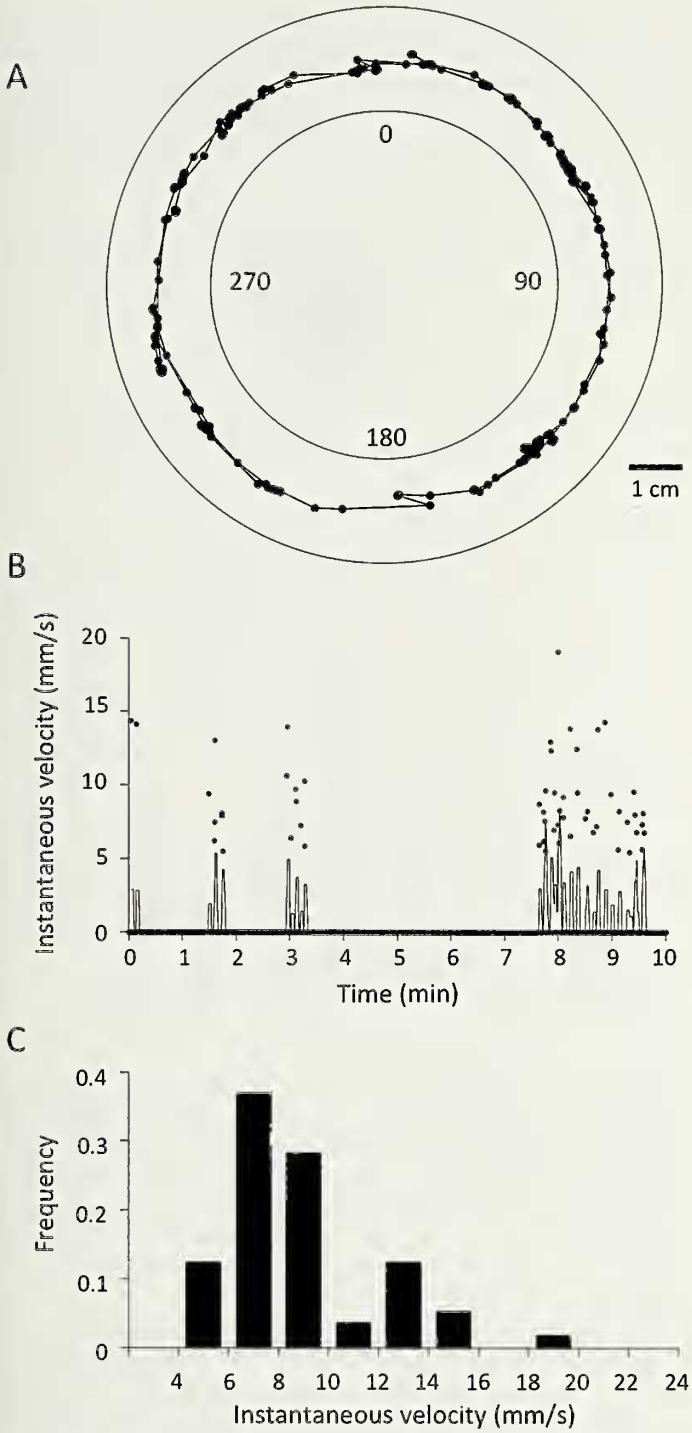

Figure 2.—Scorpion behavior in the behavioral test apparatus. **A.** Sample plot of animal movements during 10-minute trial under no light condition; the points are plotted at 0.67 s intervals. The numbers inside the circle indicate arena coordinates that are referenced in figure 3A. **B.** Plot of instantaneous velocity across duration of the trial shown in A. Instantaneous velocity is calculated as distance traveled in mm between each frame divided by 0.67 s (time between frames). The line is a five-point running average of instantaneous velocity. **C.** Frequency distribution of the instantaneous velocities for the trial shown in A.

The arenas were inserted into four holes in a 36 × 30 × 1.5 cm particleboard stage that was suspended by a PVC frame with adjustable supports for leveling. The outside upright walls of the larger Petri dish arenas were covered by black electrical tape to keep light from entering the sides of the arenas and to help the dishes fit snugly in the stage holes. The dishes were lowered into the holes until the arena lids rested flush against the top of the particleboard stage. We then placed a piece of PVC pipe (10 cm diameter and 15 cm tall) lined with black construction paper over each arena and topped this pipe with a black square of Plexiglas that had a 5-mm hole drilled in its center to accommodate an LED light. We fitted four such tubes with LEDs emitting UV light (395 nm, 15° viewing angle; Super Bright LEDs Inc.). The LEDs were fixed in place with black electrical tape and connected via patch cables with mini-hook clips to op-amp circuits to control the intensity of each LED and allow the light of each arena to be independently set, so that each trial could include four separate treatments. We filmed the arenas from below with an infrared-sensitive camera (Sony Handycam CCD-TRV16 with 'nightshot' feature) connected to a computer running a video capture program (Elgato Video Capture System). To reduce glare and improve the image, we covered the camera's IR light source with two layers of black electrical tape. We directed the IR light emitted from two surveillance cameras (Swann NightHawk Day/Night Security Cameras) at 45-degree angles onto the bottom of the arenas to provide the IR source for the camera. We taped some thin semi-transparent foam packing material over the lights to diffuse the IR. To reduce glare and light contamination of the video image from the trial LEDs, we taped a circle of black construction paper to the bottom of the arena to cover the area occupied by the smaller Petri dish.

Light calibrations.—We used an Ocean Optics USB4000-UV-VIS-ES spectrophotometer (200 μm slit, 600 μm diameter optical fiber, 3900 μm diameter CC-3 cosine corrector) to calibrate each LED to the same relative irradiance for each trial. Since we exposed the animals to LEDs emitting only UV, we matched the irradiance of these LEDs to the isolated UV component of natural light levels such as moonlight and sunlight.

We tested the scorpions' responses to four light levels. The highest level, 0.15 μW/cm^2, is approximately equal to the ultraviolet component of sunlight when the sun is just over 11.4° above the horizon; this level is also about 1500 times the ultraviolet component of full moonlight (0.0001 μW/cm^2). This allowed us to verify that our scorpions demonstrated behavior similar to that observed under previously tested light conditions (Gaffin et al. 2012). We compared the scorpions' behavior under this light to their behavior under the UV irradiances of sunset (light emitted from the sky when the sun is at the horizon; 0.01 μW/cm^2), full moonlight and no light. We determined these intensities based on irradiance values given in Figure 2C of Johnsen et al. (2006) and conversion factors provided by Johnsen (2012).

Dose response trials.—We conducted these trials in a windowless room at the Sevilleta field station in September 2012. All animal manipulations were done under dim red light provided by a headlamp (Energizer Trailfinder 6 LED Headlight); previous studies showed no apparent behavioral sensitivity of scorpions to red light (Camp & Gaffin 1999;

Gaffin et al. 2012). Trials began around 2000 (30 min after the beginning of the dark cycle) and were completed by 2130. Trials were run four at a time, with each arena's light tuned to one of the four light levels: arena A, no light; arena B, 0.0001 $\mu W/cm^2$; arena C, 0.01 $\mu W/cm^2$; arena D, 0.15 $\mu W/cm^2$. Based on the activity levels of *P. utahensis* under these conditions in our pilot studies and as reported in previous studies (Blass & Gaffin 2008; Gaffin et al. 2012), 24 animals participated in two sets of trials separated by 15 days. Within each set of trials, each animal experienced a different intensity on four successive nights. The light intensity order was randomized so that the animals were exposed to neither ascending nor descending intensities; the sequence was reordered for the second set of trials. Each animal was therefore exposed to each light condition twice, separated by about 15 days. One animal died during the 15-day interval and was removed from all data analyses. Subtracting this animal, 184 trials formed the data set for these experiments (23*4 + 23*4). Each animal was fed a wax worm seven days before the start of the first set of trials and another worm one day after completing the first set of trials.

The protocol for all trials was identical. Each night, the 24 animals were lined up in their numbered containers on the counter in the dark room. Five minutes before the beginning of the each trial, the four arenas were cleaned with 70% ethanol and dried with a Kimwipe, to ensure that the animals could not detect pheromones or other clues from previous animal use. One animal was then put into each arena, and the lids were secured with two small strips of electrical tape. The arenas were then placed in the holes on the particleboard platform under the PVC pipe with the correct light intensity, but with the arena lights switched off. Once all four animals were in place, the video and the lights were turned on. The video was set to turn off automatically after 10 minutes. Halfway through one trial, we set up the arenas for the next trial. When the video turned off, the arena lights were switched off, the video was saved to the hard drive, and the four trial animals were returned to their home containers. The next video was readied and the four new animals were placed on the stage under the correct lights. This routine was completed for all 24 animals (six groups of four simultaneous trials per night).

Analysis.—We imported each video (saved in .mp4 by the Elgato system) to iMovie (Apple Corporation), used the speed function to increase the playback rate 10×, and saved to .mov format (Quicktime, medium band). These clips were imported into ImageJ (Rasband 2012), and the images were cropped to 100 × 100 pixel squares around each of the four arenas; each cropped image was saved to a separate animated .tif file. Each file was then imported to Fiji (Schindelin et al. 2012) and converted to 8-bit format and adjusted with the Image–Adjust–Threshold function to highlight and extract the scorpion from the background. Then the outside and the inner circle areas were cleared to isolate the image to the scorpion track. We further cleaned the image by digitally removing small video incongruities outside areas of animal movement. We used Fiji's Mtrack2 plugin to track scorpion movements (settings: minimum object size (pixels) = 50; maximum object size (pixels) = 99999; maximum velocity = 50; minimum track length (frames) = 2). The software marked the position of a centroid defined by the animal's outline. If there was too much glare contamination for accurate automated tracking (about 25% of the trials), we tracked the animals manually using Fiji's Manual Tracking plugin, marking each point based on a position just caudal to the medial eyes. After processing, the ten-minute videos parsed to 900 frames in Fiji. Therefore, each frame represented 0.67 seconds (600 s / 900 frames).

We imported each of the tracked files to Excel for further analysis. First, we applied the Pythagorean theorem to calculate the distance (d) moved between each frame in the record:

$$d = \sqrt{\left((x_2 - x_1)^2 + (y_2 - y_1)^2\right)}$$

(where $[x_2, y_2]$ and $[x_1, y_1]$ are the scorpion's coordinates within the current frame and the previous frame, respectively). We then removed all movements less than 4 pixels (3.6 mm; 1 pixel = 0.9 mm) between frames to avoid bias from some jitter introduced by the automatic tracking program. We then summed the remaining distances to obtain the total distance moved for the trial. We set a threshold of at least 100 total pixels (9 cm) moved for trials to be considered legitimate; this distance is approximately two-thirds of the way around the arena track and is roughly equivalent to the movement criterion used in Gaffin et al. 2012. Scorpions typically show bouts of movement interrupted by prolonged pauses. This minimum distance was important to filter random movements from potential stimulus-induced responses.

Next we calculated the instantaneous velocity of each movement by dividing each legitimate distance (those > 4.0 pixels between frames) by 0.67 s (the time between frames). We used these numbers to derive a frequency plot of all of the instantaneous velocities for each trial. A plot of all 5648 instantaneous velocities obtained during this study shows a positive skew (mean = 10.91, median = 9.58, mode = 5.54, standard deviation = 5.18; Pearson's first and second skewness coefficients: 1.04 and 0.77, respectively). Because of the skewed distributions, we calculated the median instantaneous velocity for each trial and used those numbers to determine the mean of each animal's scores for legitimate trials for each light level. For a given light level, if an animal had one trial that was legitimate and another that was not, the legitimate score was used as the animal's seore. No score was given if neither trial was legitimate; these trials were not included in our statistical analyses.

Note that the scorpions' behavior ean be described in terms of either the total distance traversed or the instantaneous velocity. In our experience, measuring the total distance traversed is unsatisfactory, as control animals often walk slowly and deliberately, covering the same distance overall as stimulated animals that "sprint" and rest. In this study, distances traveled by animals exposed to different light levels were not significantly different (repeated measures ANOVA: Fr = 1.000; $P = 0.8438$); we therefore estimated aetivity levels based on instantaneous velocity.

We used circular statistics to test for bias in the animal arena position in these trials. We first calculated the mean vector direction for animal positions in each legitimate trial. We then used these directions to calculate the overall mean vector direction and length (r). We calculated the z-statistic and used the Rayleigh test for randomness to determine the statistical significance of the mean vector.

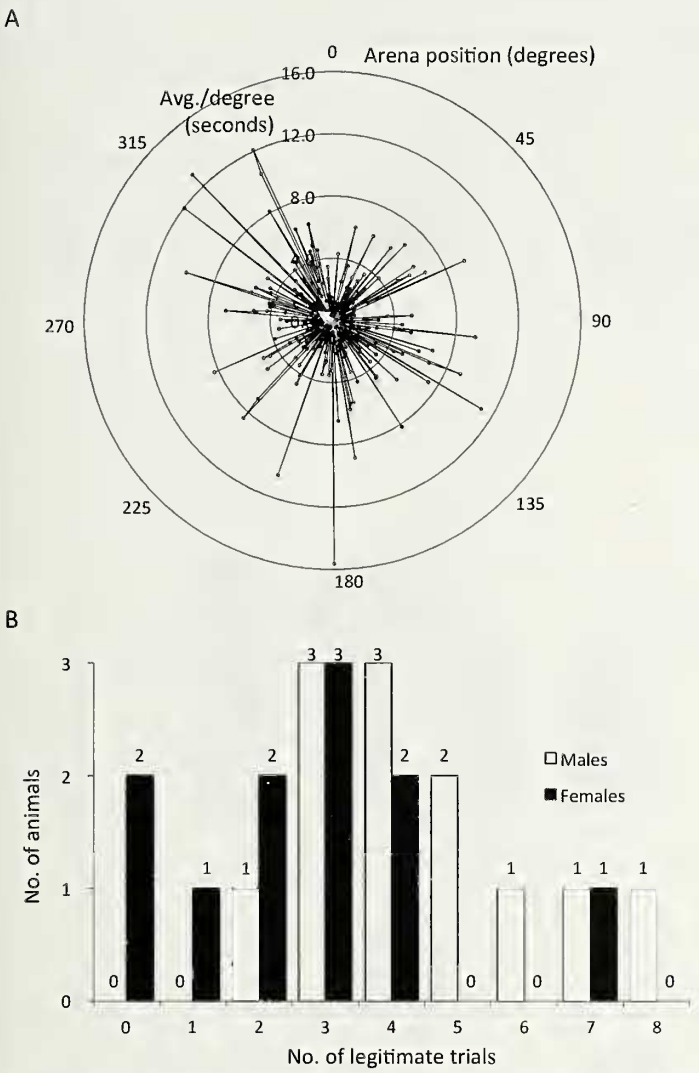

Figure 3.—Activity patterns in behavioral apparatus. **A**. Average animal arena position (in degrees) for all 83 legitimate trials shows no arena or global position bias (0 degrees is toward the top of the video screen; 290 degrees is geomagnetic north relative to this reference). **B**. Females and males show differences in the number of trials that met the legitimacy criterion.

We also looked at activity differences between males and females. We used a two-tailed Mann-Whitney U analysis to test statistical differenees in number of legitimate trials per animal among males and females.

We used a repeated-measures ANOVA and Dunn's multiple eomparisons post-hoc test to analyze the significance of differences among the scorpions' median instantaneous velocities under different light intensities. In this assay, scorpion responses appear as spurts of locomotor movement; as such, we predicted that stimulated scorpions would have higher median instantaneous velocities. We considered treatments significantly different if the P value was less than 0.05. Our null hypothesis was that there would be no significant differences between the scores at different irradiances. We used InStat 3 statistical software (Graph Pad Software, Inc., San Diego, CA, U.S.A.) for the Mann-Whitney U and ANOVA analyses.

Table 1.—Number of legitimate trials by experimental factor.

Factor	Condition	Legitimate trials	Total trials
Time of night	Early	48	96
	Late	35	88
Trial set	First	39	92
	Second	44	92
Night order	1	22	46
	2	21	46
	3	21	46
	4	19	46
Gender	Males	54	96
	Females	29	88

RESULTS

The new behavioral apparatus is different from the one used by Blass and Gaffin (2008) and Gaffin et al. (2012) in that the Petri dish arenas are suspended in holes in a particleboard stage rather than sitting atop a Plexiglas stage. The arrangement we used in this study, coupled with diffuse IR light directed from the side, provided clear images of the scorpions when filmed through the Petri dish from below. The scorpion images were distinct enough to use video detection software to automatically track animal movements, thereby removing the potential bias of a human observer. A sample plot of an animal moving under the "no light" condition is shown in Fig. 2A. The time course of this animal's instantaneous velocities is shown in Fig. 2B and these data are compiled in Fig. 2C as a frequency plot of the instantaneous velocities for the 10-min trial.

We made various checks of the new behavioral assay. Legitimacy (> 9 cm movement) was achieved in 45.1% of the trials (83 of 184). Although we recognize that considering all 83 legitimate trials includes non-independent observations, we feel that we can learn something about general patterns of behavior by examining these data. We found no bias in arena or global position among the legitimate trials (Fig. 3A: φ = 307°, r = 0.0369, z = 0.1132. P = 0.8935). Table 1 gives the number of legitimate trials by time of night (roughly, first half from 2000 to 2045, second half from 2045 to 2100), trial set, trial night and gender. Pooling across all light conditions, males had more legitimate trials than females (Fig. 3B: P = 0.0265, Mann-Whitney, two-tailed; U-statistic = 30.0).

Figure 4 shows an example of an animal with legitimate trials under all four light levels. This composite shows a general trend in behavior, with animals under no light (4A) or the lowest light (0.0001 $\mu W/cm^2$; 4B) conditions making shorter, steadier movements than the sporadic movements of animals under the highest light (0.15 $\mu W/cm^2$) condition (4D). The 0.15 $\mu W/cm^2$ trial contained the highest proportion of instantaneous velocities greater than 28 mm/s (right side of 4D). Likewise, the 0.01 $\mu W/cm^2$ trials occasionally contained examples of faster instantaneous velocities, as can be seen in the initial movements depicted in the middle plot of 4C.

The averages of instantaneous velocities for scorpions under each light level for all legitimate trials are shown in Fig. 5A. The graph shows similar patterns for the no light and 0.0001 $\mu W/cm^2$ trials and a flattening of the distribution pattern for the 0.15 $\mu W/cm^2$ trials. The pattern for the

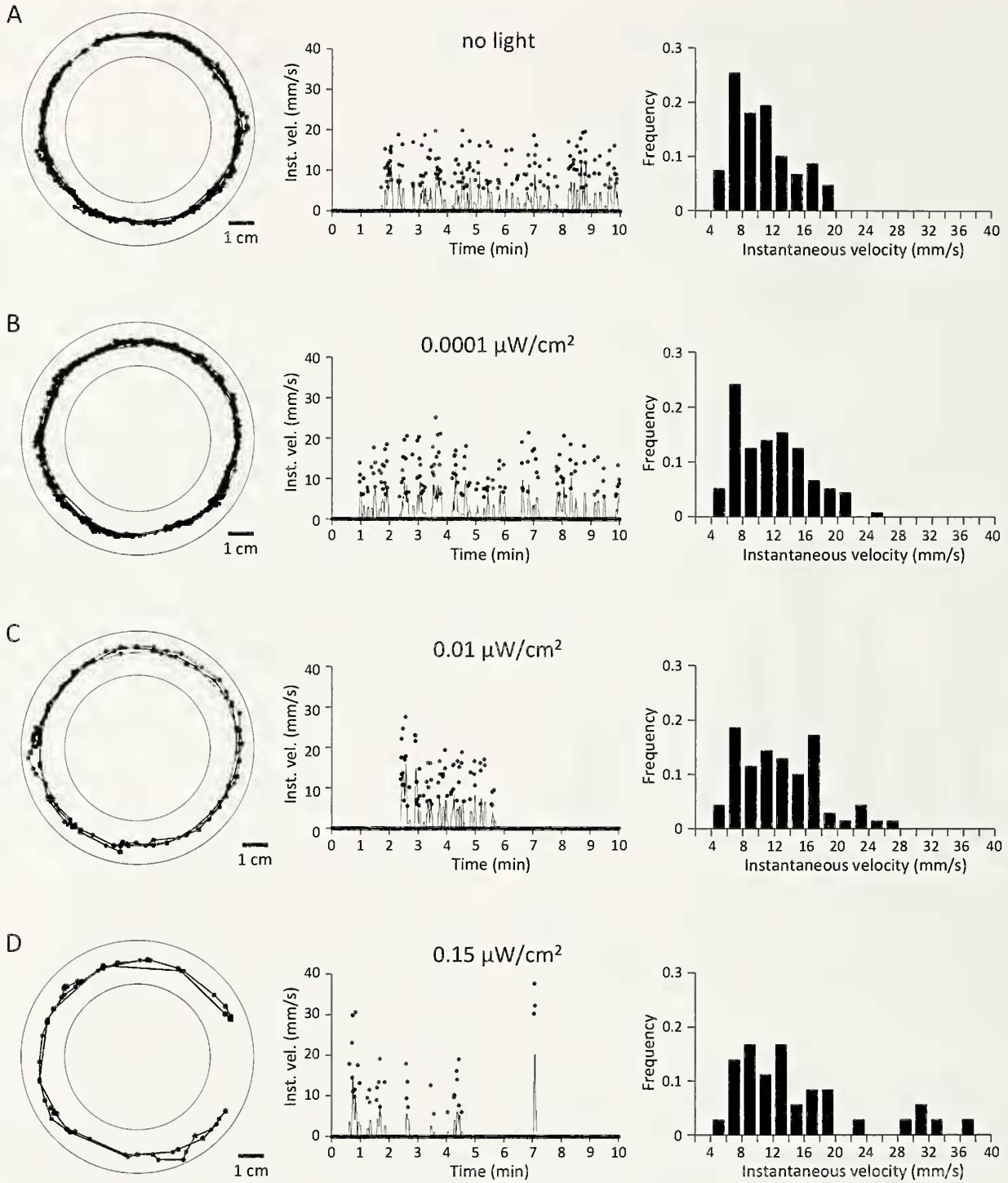

Figure 4.—Sample behavioral response of an animal to all four light levels. **A.–D.** Response to no light, 0.0001 µW/cm^2, 0.01 µW/cm^2 and 0.15 µW/cm^2, respectively. Left: plots of animal movements in arena; middle: plots of instantaneous velocities during the 10-min trials (lines = five-point running averages); right: frequency distributions of instantaneous velocities.

0.01 µW/cm^2 trials is similar to, but slightly below, the no light and 0.0001 µW/cm^2 trials curves.

Figure 5B shows the distribution of the 83 legitimate trials based on light treatment. We scored each animal as 0 if neither its first nor its second trial was legitimate, 0.5 if one of its two trials was legitimate, and 1 if both of its trials were legitimate. A Friedman test (nonparametric repeated measures ANOVA) across these data showed significant variation among the treatments (P = 0.0274); Dunn's multiple comparisons test showed no significant difference between pairs of treatments. Figure 5C compares the median instantaneous velocity scores among the light levels (scores were averaged for animals with two legitimate trials within a given treatment). Seven of the 23 animals had legitimate trials across all four treatments.

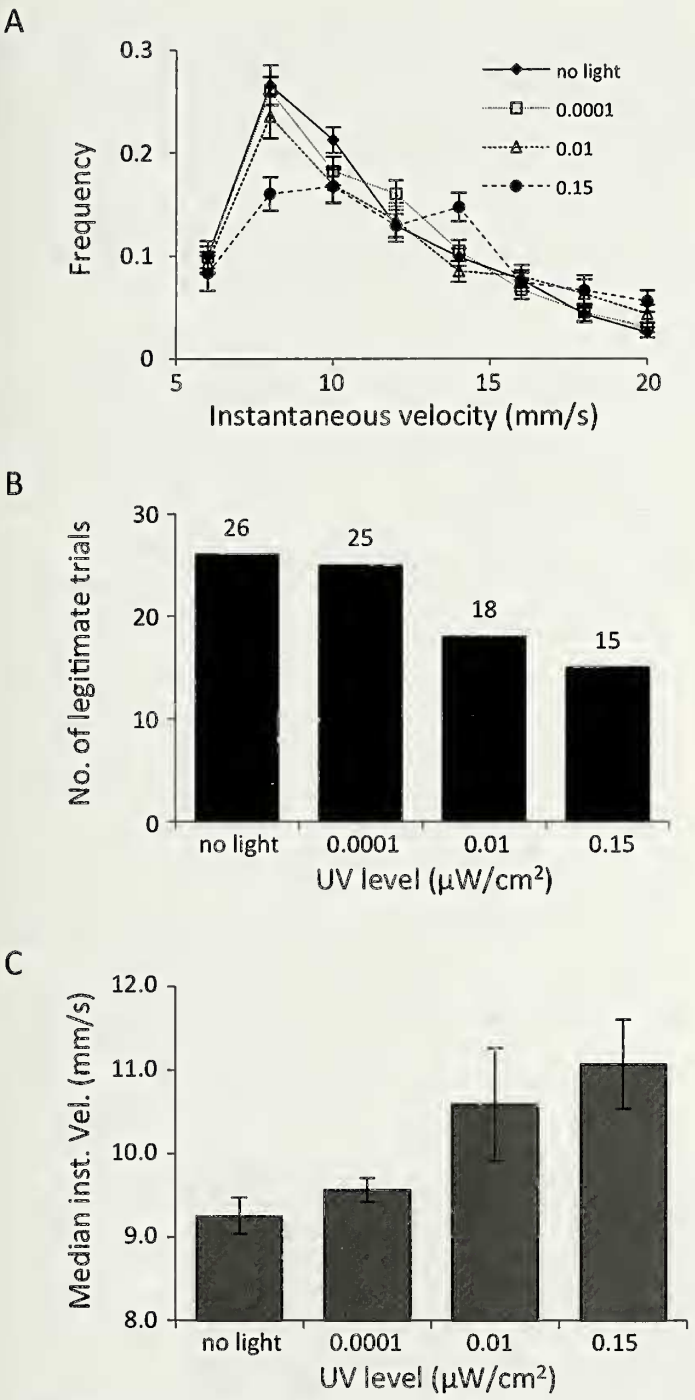

Figure 5.—Composite behavioral responses to various UV levels. **A**. Averaged instantaneous velocity distributions for the four experimental light levels (mean ± SE). **B**. Number of legitimate trials parsed by light level. **C**. Median instantaneous velocities by light level for the seven animals included in our repeated measures ANOVA analysis (mean ± SE).

However, the no-light values for one animal were more than two standard deviations greater than the mean; we therefore removed all trials for this animal from our analyses, reducing our sample size to six. A repeated measures ANOVA showed significant overall differences among the light levels ($Fr = 9.000$; $P = 0.0218$); there was also a pair-wise difference between the 0.15 μW/cm^2 and no-light treatments ($P < 0.05$).

DISCUSSION

The results of these studies are clear: using a negative phototactic behavioral assay, scorpion locomotor behavior is highest at UV irradiance levels that correspond to sunset. Adding additional indicators, such as the distribution of instantaneous velocities and the number of legitimate trials by light treatment, suggests that the UV response threshold detected by this assay is between the 0.15 μW/cm^2 and 0.01 μW/cm^2 light responses. Pooling across all light treatments, we also found a difference in the activity of males and females, which is to be expected since these trials were conducted at the end of the mating season when males are more active on the surface and tracking females (Bradley 1988).

This study demonstrates that scorpions respond differently to different UV levels experienced during the normal activity time of *P. utahensis*, the time at sunset when they normally move to the thresholds of their burrows (Gaffin 2011). Since the assay used in these trials measures scorpion locomotor behavior, the absolute detection sensitivity to UV could be much lower. In addition, these studies suggest that relatively high UV inhibits normal scorpion locomotion, indicated by the low number of legitimate trials under 0.15 μW/cm^2 and 0.01 μW/cm^2 levels. This inhibition affects the sensitivity of the assay because many animals did not move at all during the 10-min trials at high UV irradiation. Those data were dropped from the analyses (59 of 92 trials = 64%).

Several recent studies suggest that scorpion cuticle plays a role in UV detection (Kloock et al. 2010; Camp & Gaffin 1999; Blass & Gaffin 2008; Gaffin et al. 2012). Kloock et al. (2010) found that scorpions that had their fluorescence compromised by photo-bleaching made more transitions between UV light exposed and unexposed regions of Petri dish arenas than untreated scorpions; also, fluorescent scorpions reduced their activity under UV light at intensity levels similar to what we present here. The authors discuss the possibility that scorpion fluorescence is related to the detection of moonlight and the decision to avoid foraging on nights with high moon illumination; scorpions are less active on the surface during moonlit nights than during moonless nights (Skutelsky 1996). However, our results do not support this notion since animals under UV levels that match the UV composition of full moon nights showed no difference in behavior from animals under no-light conditions. This does not mean that the animals are not detecting and using UV at full moon levels; it simply suggests that it does not act as a deterrent.

Gaffin et al. (2012) found similar locomotor responses of scorpions under UV and green wavelengths at the 0.15 μW/cm^2 intensity and differences in behavior under the two wavelengths when the eyes were covered. The behavior of eyes-blocked animals changed more when exposed to green light than to those animals exposed to UV light, suggesting a possible role for the fluorescent cuticle in UV detection. Gaffin et al. (2012) suggested the cuticle could serve as a whole-body light detector for purposes of finding shelter. That is, shading of any part of the cuticle stimulated by UV would represent overhead shelter (such as a twig or blade of grass), and a reflexive turning toward the shaded side would move the animal's body under the shelter.

We made several changes to earlier behavioral assay protocols to improve the efficiency of the trials. Most

significantly, we dramatically improved the scorpion image by removing interference from the Plexiglas stage and glare from direct IR projection of the recording camera. By using diffuse IR from the side, the image cleared to a point that we could easily detect scorpions using the public domain ImageJ image-processing program. Once resolved from the background, the animals were accurately tracked via Fiji's Mtrack2 plugin. Automated tracking greatly reduces scoring time and removes the potential for human bias. We think similar tracking will be useful for additional scorpion studies, including those behaviorally testing for and identifying chemicals that make up scorpion pheromone secretions (Taylor et al. 2012).

Additional steps need to be taken to determine whether scorpion fluorescence has an adaptive function in UV detection. Although our assay has been useful for detecting a response and a potential deterrence threshold, it is also laborious, time consuming and requires a large number of trials to register an effect. It would be helpful to develop a behavioral assay that measures individual responses to light of various intensities and wavelengths, perhaps focused on various body regions. Also, it could be useful to reduce the fluorescence through bleaching (Kloock 2009) or other means to see if the behaviors we observe can be compromised. Finally, some members of the family Chaerilidae Simon have been recently reported to lack the fluorescence phenomenon (Lourenço 2012). These animals could be useful in comparative light detection assays with normally fluorescent animals.

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LITERATURE CITED

Blass, G.R.C. & D.D. Gaffin. 2008. Light wavelength biases of scorpions. Animal Behaviour 76:365–373.

Bradley, R.A. 1988. The influence of weather and biotic factors on the behaviour of the scorpion (*Paruroctonus utahensis*). Journal of Animal Ecology 57:533–551.

Camp, E.A. & D.D. Gaffin. 1999. Escape Behavior mediated by negative phototaxis in the scorpion *Paruroctonus utahensis* (Scorpiones, Vaejovidae). Journal of Arachnology 27:679–684.

Gaffin, D.D. 2011. *In situ* infrared videography of sand scorpion nighttime surface activity. Euscorpius 122:1–13.

Gaffin, D.D., L.A. Bumm, M.S. Taylor, N.V. Popokina & S. Mann. 2012. Scorpion fluorescence and reaction to light. Animal Behaviour 83:429–436.

Fasel, A., P.-A. Muller, P. Suppan & E. Vauthey. 1997. Photoluminescence of the African scorpion *Pandinus imperator*. Journal of Photochemistry and Photobiology B: Biology 39:96–98.

Fleissner, G. & G. Fleissner. 2001. Night vision in desert scorpions. Pp. 317–324. *In* Scorpions 2001; In Memoriam Gary A. Polis. (V. Fet & P.A. Selden, eds.). British Arachnological Society, Burnham Beeches, Bucks.

Frost, L.M., D.R. Butler, B. O'Dell & V. Fet. 2001. A coumarin as a fluorescent compound in scorpion cuticle. Pp. 363–368. *In* Scorpions 2001; In Memoriam Gary A. Polis. (V. Fet & P.A. Selden, eds.). British Arachnological Society, Burnham Beeches, Bucks.

Johnsen, S. 2012. The optics of life: a biologist's guide to light in nature. Princeton University Press, Princeton, New Jersey.

Johnsen, S., A. Kelber, E. Warrant, A.M. Sweeney, E.A. Widder & R.L. Lee Jr. et al. 2006. Crepuscular and nocturnal illumination and its effects on color perception by the nocturnal hawkmoth *Deilephila elpenor*. Journal of Experimental Biology 209:789–800.

Kloock, C.T. 2005. Aerial insects avoid fluorescing scorpions. Euscorpius 21:1–7.

Kloock, C.T. 2008. A comparison of fluorescence in two sympatric scorpion species. Journal of Photochemistry and Photobiology B: Biology 91:132–136.

Kloock, C.T. 2009. Reducing scorpion fluorescence via prolonged exposure to ultraviolet light. Journal of Arachnology 37:368–370.

Kloock, C.T., A. Kubli & R. Reynolds. 2010. Ultraviolet light detection: a function of scorpion fluorescence. Journal of Arachnology 38:441–445.

Lourenço, W.R. 2012. Fluorescence in scorpions under UV light; can chaerilids be a possible exception? Comptes Rendus Biologies 335:731–734.

Machan, L. 1968. Spectral sensitivity of scorpion eyes and the possible role of shielding pigment effect. Journal of Experimental Biology 49:95–105.

Polis, G.A. 1980. Seasonal patterns and age-specific variation in the surface activity of a population of desert scorpions in relation to environmental factors. Journal of Animal Ecology 49:1–18.

Rao, G. & K.P. Rao. 1973. A metasomatic neuronal photoreceptor in the scorpion. Journal of Experimental Biology 58:189–196.

Rasband, W.S. 2012. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA. Online at http://imagej.nih.gov/ij/

Schindelin, J., I. Arganda-Carreras, E.E. Frise, V. Kaynig, M. Longair & T. Pietzsch et al. 2012. Fiji: an open-source platform for biological-image analysis. Nature Methods 9:676–682.

Skutelsky, O. 1996. Predation risk and state-dependent foraging in scorpions: effects of moonlight on foraging in the scorpion *Buthus occitanus*. Animal Behaviour 52:49–57.

Stachel, S.J., S.A. Stockwell & D.L. Van Vranken. 1999. The fluorescence of scorpions and cataractogenesis. Chemical Biology 6:531–539.

Taylor, M.S., C. Cosper & D.D. Gaffin. 2012. Behavioral evidence of pheromonal signaling in desert grassland scorpions, *Paruroctonus utahensis*. Journal of Arachnology 40:240–244.

Wankhede, R.A. 2004. Extraction, isolation, identification and distribution of soluble fluorescent compounds from the cuticle of scorpion (*Hadrurus arizonensis*). M.S. thesis, Marshall University, Huntington, West Virginia.

Zwicky, K.T. 1968. A light response in the tail of *Urodacus*, a scorpion. Life Sciences 7:257–262.

Zwicky, K.T. 1970a. The spectral sensitivity of the tail of *Urodacus*, a scorpion. Experientia 26:317.

Zwicky, K.T. 1970b. Behavioral aspects of the extraocular light sense of *Urodacus*, a scorpion. Experientia 26:747–748.

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